EGG ALBUMEN AS A CULTURE MEDIUM FOR CHICK TISSUE.

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Egg albumen as a culture medium for chick tissue *in vitro* has received but scant attention from experimentalists, in spite of the fact that it forms the natural medium, in part at least, of the embryo chick. In a recent series of experiments, however, results have been obtained which show that all the usual manifestations of cell activity, noted by various observers in other culture media, were to be met with in cultures made from egg albumen, and have, I believe, demonstrated satisfactorily its entire adaptability to that use. These experiments were carried on in the laboratory of Prof. S. J. Holmes, to whom my thanks are due for his kindness in giving advice and assistance throughout the course of the work.

The technique followed has been that outlined by Burrows and Carrel, modified to suit the different conditions under which the work had to be carried on, using embryos varying in age from twenty-four hours to fourteen days. Of these it was found that the most successful results were obtained from embryos of from ten to fourteen days growth, though all showed considerable activity. Fragments of all the organs of the body, including the brain and spinal cord, were used, but the most active growths were obtained from the heart. Several series of preparations were made by cutting up the entire embryo into minute particles in a small amount of Ringer's solution and egg albumen, stirring and shaking these rapidly for a few minutes and then placing a small drop of the mixture on the slide and sealing in the usual way. By this process cultures could be made containing but a few or even single cells. The medium used has been egg albumen alone or mixed with varying proportions of egg yolk, Ringer's solution and extract of muscle tissue. Egg yolk proved entirely unsatisfactory because of the impossibility of seeing what was taking place within it. The best results were obtained from egg

albumen alone and with mixtures of albumen and muscle tissue extract, the latter being prepared from embryo chick tissue and added to the albumen either before or after making the culture. Egg albumen coagulates to a more or less firm consistency and thus gives one of the conditions apparently requisite for the growth and activity of the tissue cells.

Owing to the viscosity of the albumen, considerable care is necessary in handling the specimens when it becomes needful to transfer the culture to a fresh medium, the usual method of procedure being to cut away the old albumen with a sharp knife. When, as is frequently the case, the outgrowth seemed to be mainly on the surface of the glass, and thus could not be transferred in the usual way without the loss of the greater part of the growth, another method was used. Inverting the cover glass the albumen was removed with forceps and pipette, several changes of Ringer's solution successively placed over the culture and, after removal of this, a fresh drop of albumen was added to the culture and it was again sealed up.

The latent period, before the beginning of activity of the culture, lasted from half an hour to several days. Usually, in good preparation, active amœboid movements began within half an hour after being put on the slide. At that time along the border of the tissue could be seen the elongated, outpushing cells forming a fringe along what was before a clear cut outline, with a few scattered cells lying at some little distance from the main mass. These cells displayed very active amœboid movements that are less common in the older cultures though still present to some extent. When these cells are chilled or disturbed they contract and become rounded. On a number of cultures groups of cells showed long clear processes extending outward, sometimes branched, with the ends breaking up into short filaments. These were in all cases cultures which included portions of the brain or spinal cord from a four-day chick. An attempt was made to photograph one of these cultures but the length of time necessary was sufficient to chill the slide and, on examination, it was found that the processes had all been retracted. Subsequent incubation had no effect on the culture, though disintegration did not take place for several days. In all the cultures

these processes disappeared, were retracted apparently, in the course of fifty to seventy hours and no further evidences of them were seen. In the preparations made by shaking up the finely cut embryo with Ringer's solution, a greater or less number of single cells were found. In the course of a few days these were greatly increased in number with a distinct massing together of the cells, usually along the outer border of the drop of albumen. Owing to accidents of various kinds these were not carried along far enough to show the tissue formation noted by Carrel.

The most marked instance of tissue formation was that apparent in a culture made from the heart of a fourteen day chick, which, at the end of twenty days was encircled by a new formation five times the diameter of the original piece of tissue. This new formation was several cells in thickness and composed of fusiform and polygonal cells, sometimes massed together, forming a network, or in other places showing distinct cell boundaries. Among these cells many showed division figures at various stages. Around the outer margin of the mass of cells and extending nearly three-fourths of the entire distance around it, the cells had taken on a different character. Here they had become flat, thin and elongated in a direction parallel with the margin of the circle. This formation was several cells in thickness with the cells closely matted together and forming a distinct boundary that was conspicuous without the aid of a lens. The remaining one-fourth of the margin was occupied by cells actively pushing outward.

To test the effects of cold on the growth of the tissues, the embryo was sealed up in a stender dish containing Ringer's solution and placed in the ice box of the refrigerator with the temperature but a few degrees above zero, Centigrade. The first of these was used the second day and behaved like normal tissue. Most of those kept in the refrigerator for a number of days became infected with bacteria. The longest period of cold storage which gave successful cultures was four days, from January 31 to February 4. One half hour after making the cultures from this embryo the cells were moving out in an active condition in four out of the sixteen cultures made. The subsequent history of these cultures was the same as that of unrefrigerated tissue.

The longest period during which tissues have been kept alive

without any evidences of necrobiosis has been ninety-three days, and in the majority of these cases death has been caused by infection with bacteria or molds or other accidents, and, not, apparently, by any lack of vigor in the tissues themselves. This, in general, seems to be true of most of the cultures which appear to be in a thriving condition after the second day or third day, and especially where renewals of the culture medium have been frequent, and precautions have been taken to avoid tearing or otherwise injuring the tissues. However disintegration frequently takes place from no apparent cause.

Egg albumen presents some difficulties when a stained preparation from the culture is desired, on account of its avidity for stains. In the first stained preparations made it was impossible to distinguish the outlines of the cells, and the study of the specimen seemed a hopeless task. This difficulty was later overcome by the following methods: the cover glass was inverted and placed on the mouth of a vial containing a quantity of osmic acid. The mouth of the vial was small enough to be completely covered by the cover glass and yet not touch the preparation. After fixing in this manner for ten minutes the cover glass was placed in a stender dish containing distilled water and left for a number of hours. Frequent agitation and changes of the water removes the greater part of the albumen, leaving the tissue adhering to the glass, which may then be put through the alcohols and stained in the usual way. With this method very clear preparations may be obtained.

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